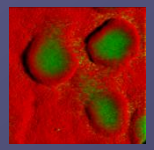
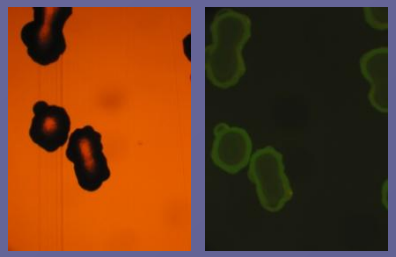
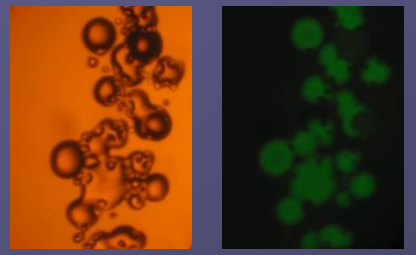


# Biofilm formation and antibiotic tolerance: is there a connection

**Anders Folkesson**

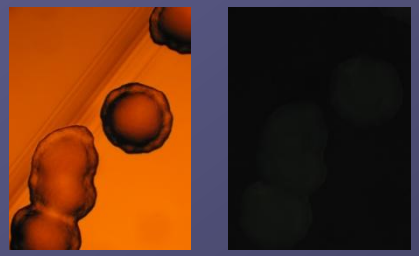
**IMG**
Infection Microbiology Group
DTU Biosys
The TECHNICAL UNIVERSITY of DENMARK



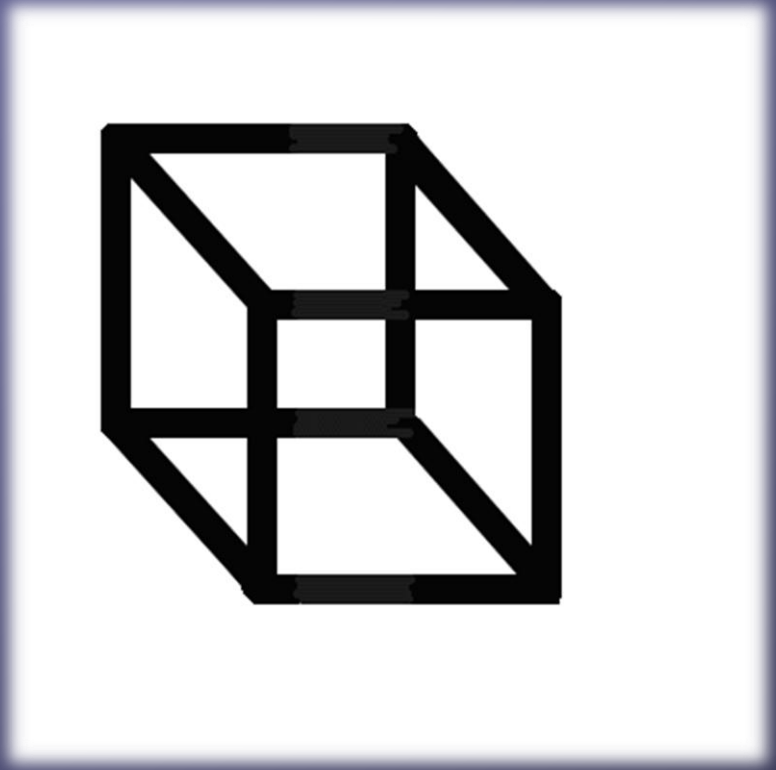


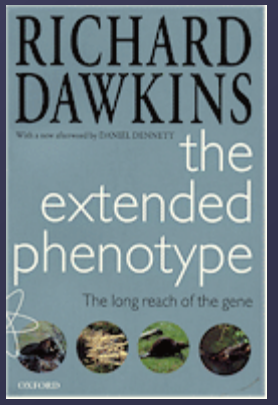
Jacob Olsen
Andreas Arnung
Christian Skjødt

J.A.J Haagensen
A.Reisner
C.Zampaloni
C. Sternberg
S.Molin



# The Necker cube.





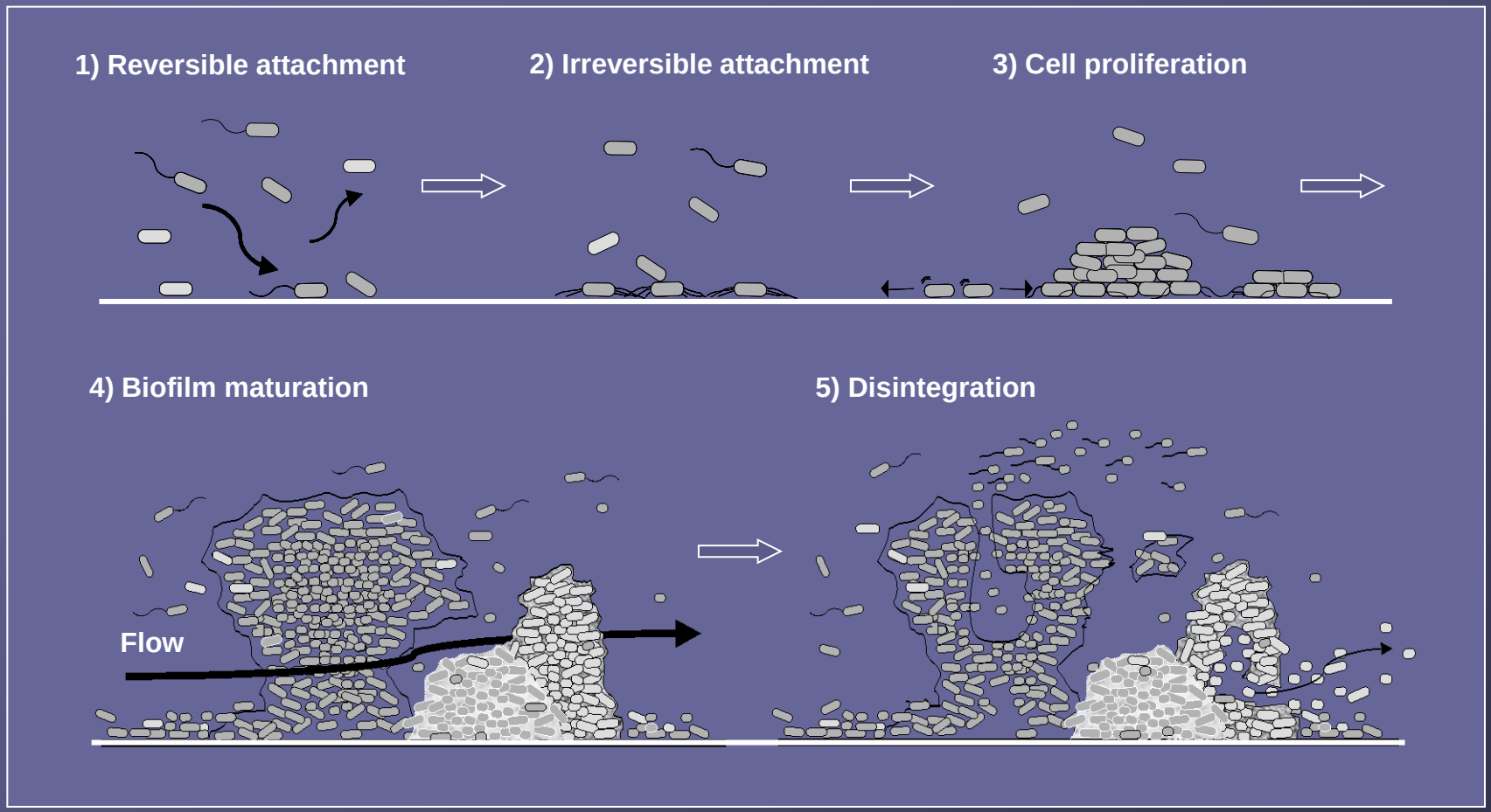
**Biofilm formation**

**Biofilms and antibiotics**

***E.coli* as a model system**

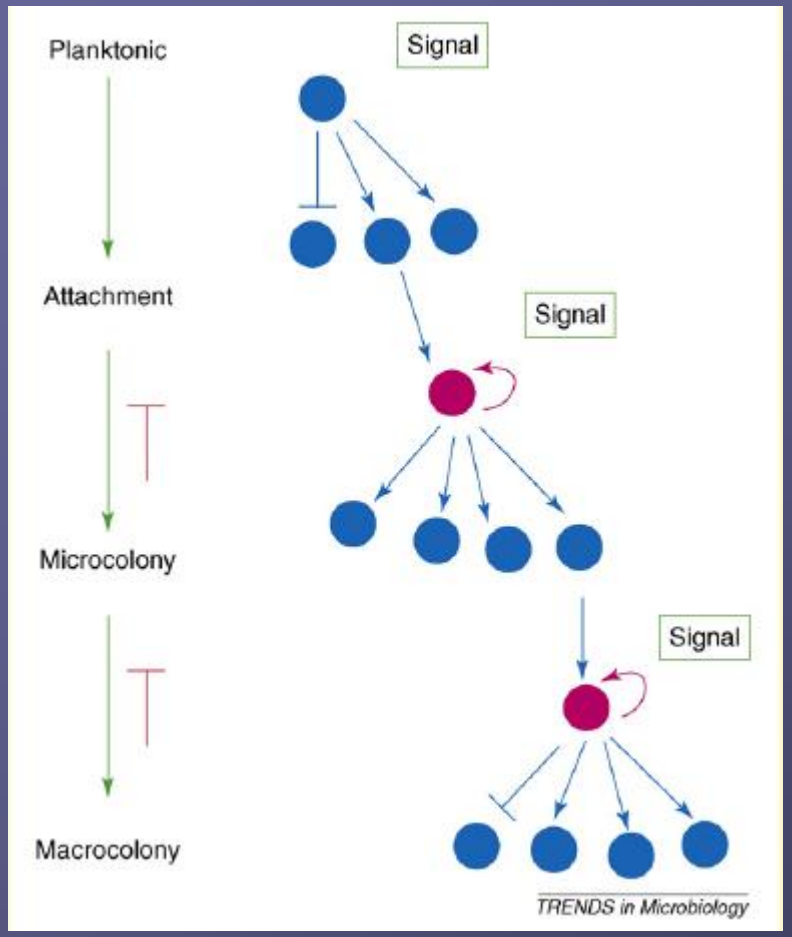
**The dynamics of Ab action in microbial biofilms**

# The classical model for biofilm formation.




A model of the stages of bacterial biofilm development. At stage 1, the bacterial cells attach reversibly to the surface. Then, at stage 2, the cells attach irreversibly, a step mediated mainly by exopolymeric substances, and the cells lose their flagella-driven motility. At the next stage (3), the first maturation phase is reached, as indicated by early development of biofilm architecture. The second maturation phase is reached at stage 4 with fully mature biofilms, as indicated by the complex biofilm architecture. At the dispersion stage (5), single motile cells (dark cells on the figure) disperse from the microcolonies

# The classical model for biofilm formation.

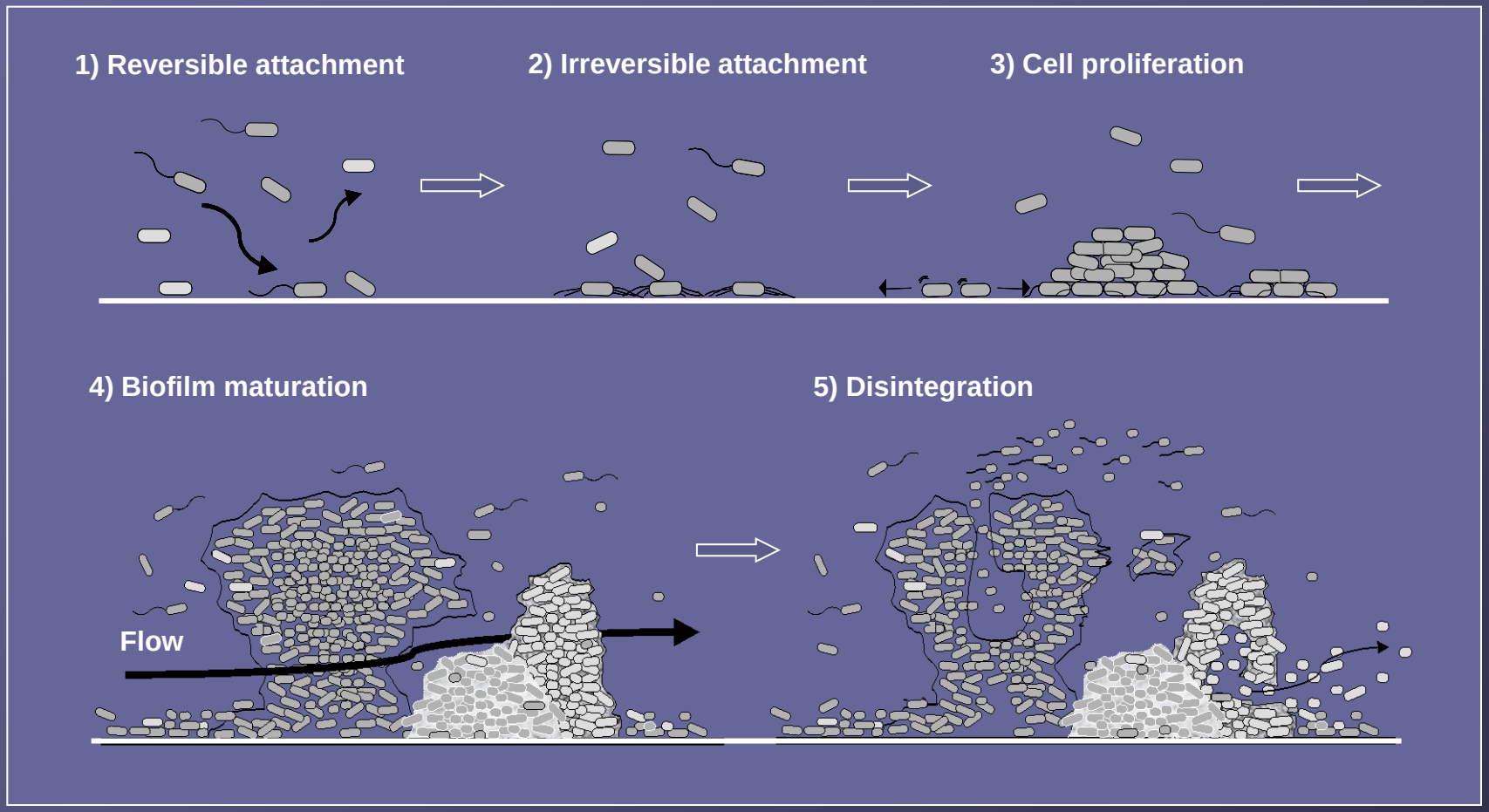



Developmental pathways have evolved as dedicated systems for regulation of biofilm formation.

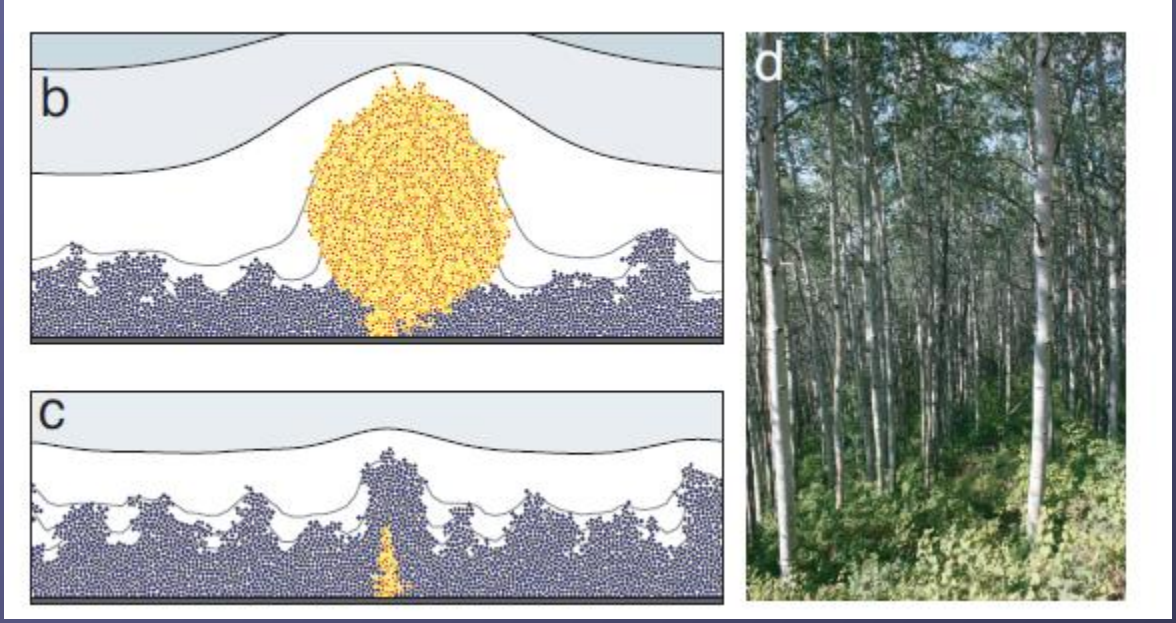
The developmental model describes biofilm formation in terms of a multicellular process, wherein genetic pathways are presumed to have evolved to facilitate cooperation among members of the biofilm

*Monds et.al.2009*

# The not so classical model for biofilm formation.



# Individual based modelling of biofilm formation.




Xavier PNAS 2007

**How can we differentiate between the two models?**

Does it matter, anyway?

# Increased tolerance to antimicrobial agents is an important feature of microbes growing in biofilms.

Infections caused by bacterial biofilms
can be persistent and difficult to eradicate.

# Susceptibility of planktonic and biofilm bacteria to selected antibiotics

| Organism | Antibiotic | Planktonic (μg/ml) | Biofilm (for 99% reduction) |
|---|---|---|---|
| *S. aureus* | Vancomycin | 2 (MBC) | 20 (μg/ml)$_{\text{siliconedisc}}$ |
| *P. aeruginosa* | Imipenem | 1 (MIC) | > 1024 (μg/ml) |
| *E. coli* | Ampicillin | 2 (MIC) | 512 (μg/ml)$_{\text{calgary}}$ |
| *P. pseudomallei* | Ceftazidime | 8 (MBC) | 800 (μg/ml)$_{\text{robbins}}$ |
| *S. sanguis* | Doxycycline | 0.063 (MIC) | 3.15 (μg/ml)$_{\text{robbins}}$ |

Adapted from Donlan *et al. Clin. Microbiol. Rev.* 2002

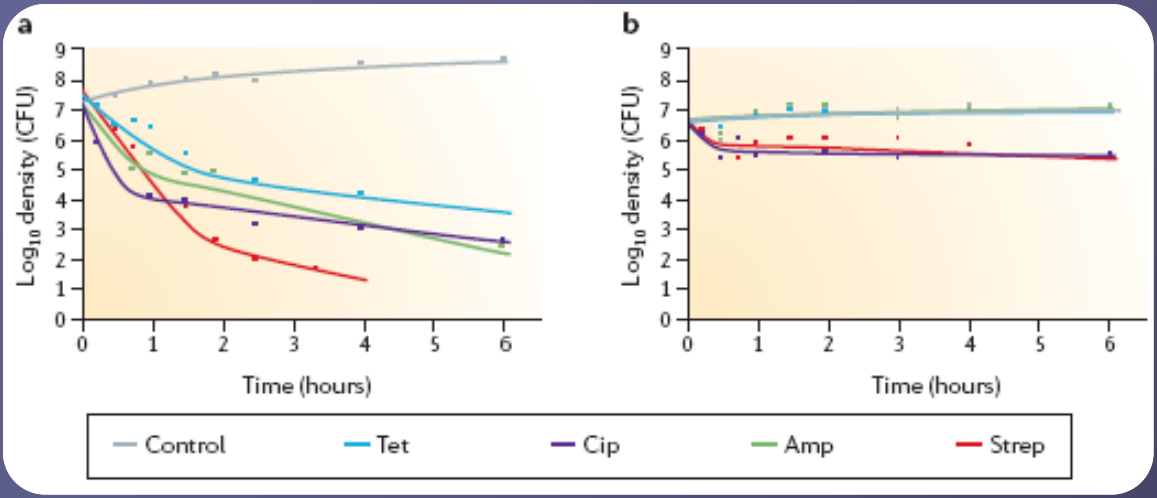
**$Ab^r$ Planktonic < $Ab^r$ device.**

**Bacteria form biofilm in device.**

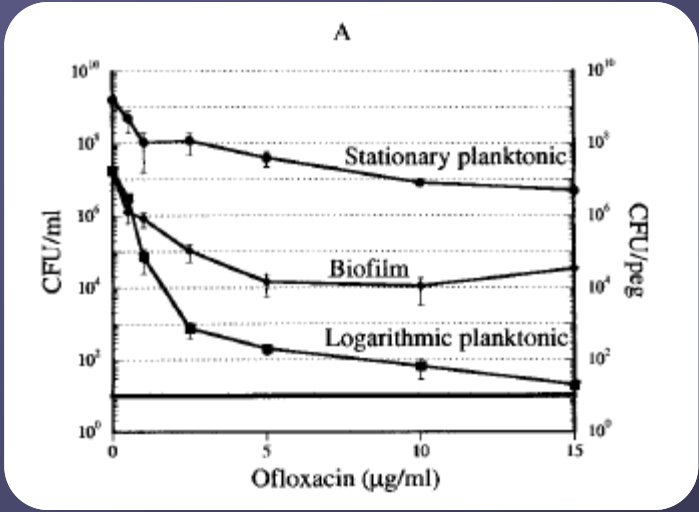
**Therefore are biofilms more resistant.**

**Is a causal relationship established?**

# Biofilm vs stationary phase as an explanation for the failure of AB treatment without resistance.

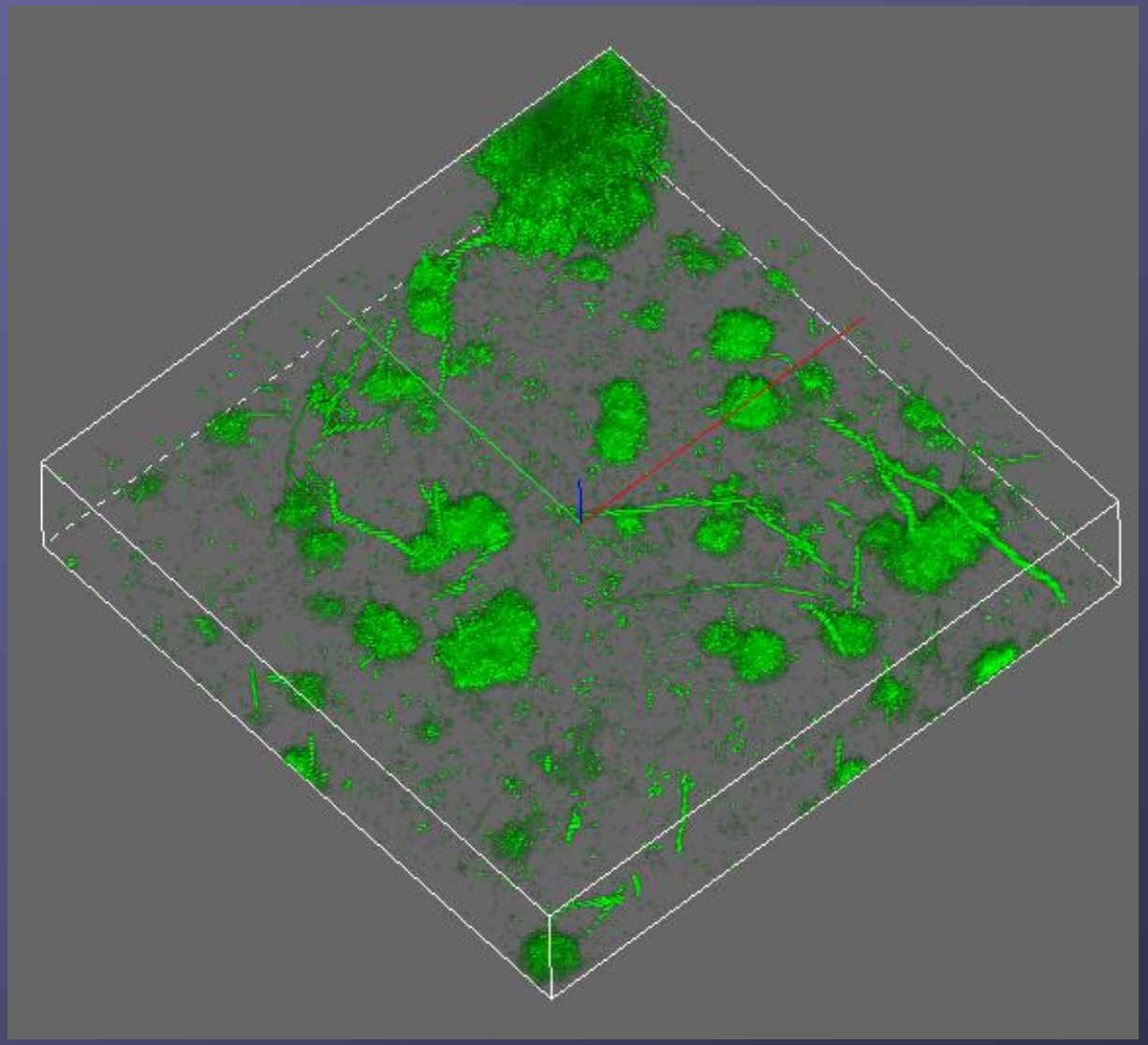



Levin et.al 2006

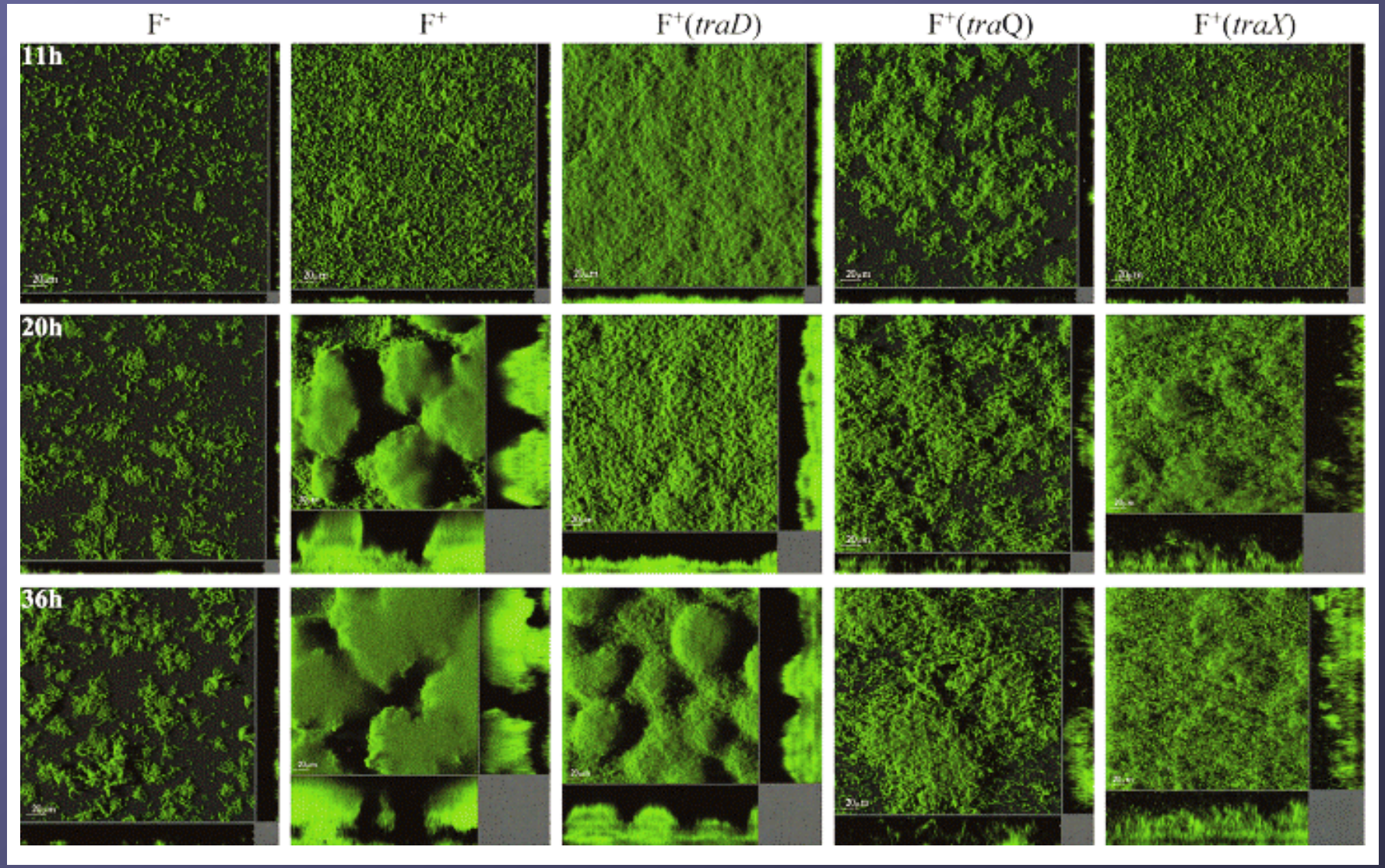



Spoering et.al 2001

# *Escherichia coli* is not an efficient biofilm former.



# The spatial distribution of *E. coli* biofilms.



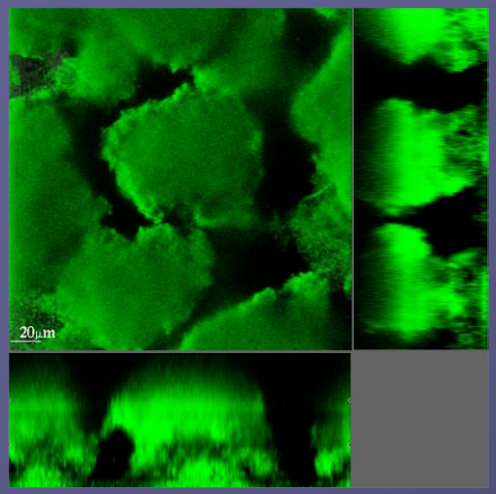

Reisner *et al. Mol Microbiol 03*

**The only factor needed for induction of the mushroom state is the pili.**

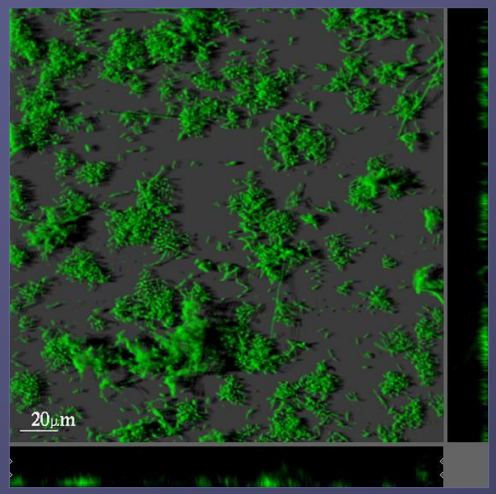
**A set of isogenic strains.**

**Biofilm effect under identical conditions.**

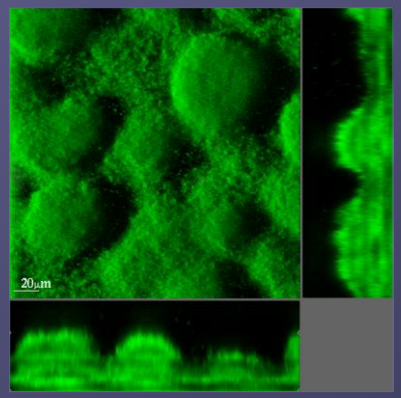
**F$^+$**



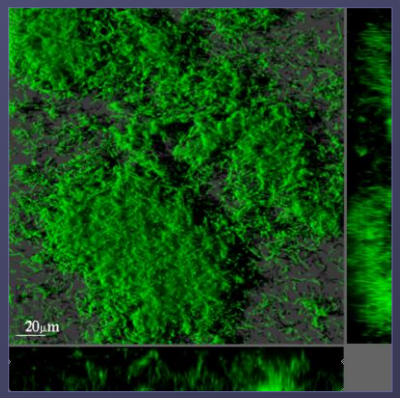

**F*traA***



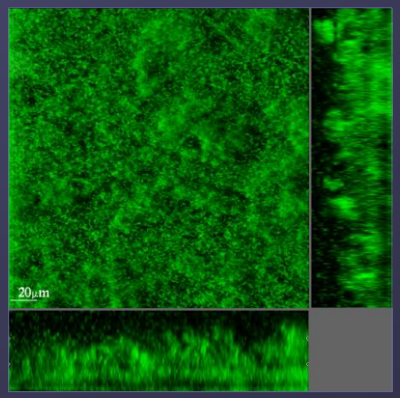

**F*traD***



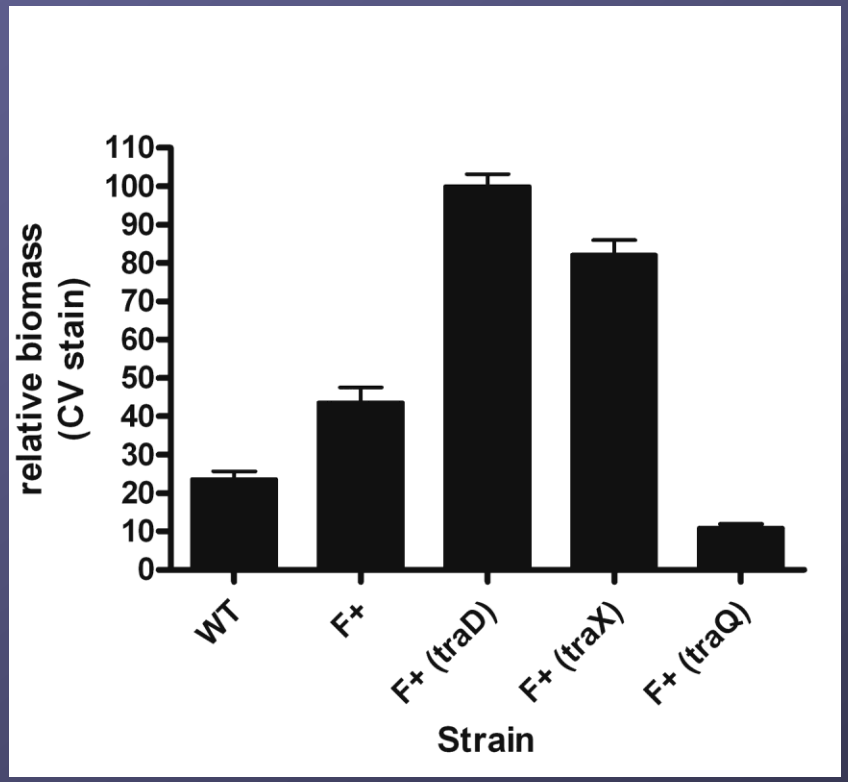

**F*traQ***



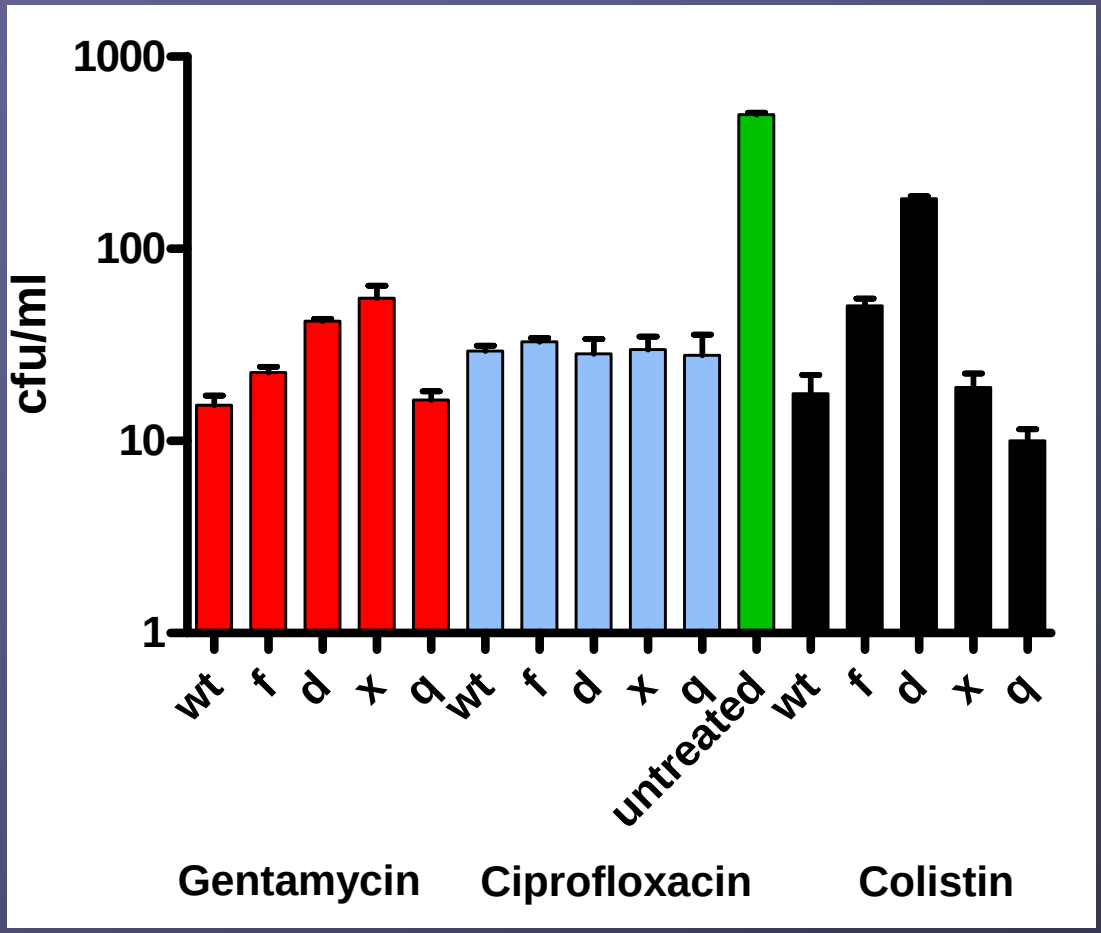

**F*traX***



# Microtiter biofilm assay CV-staining.



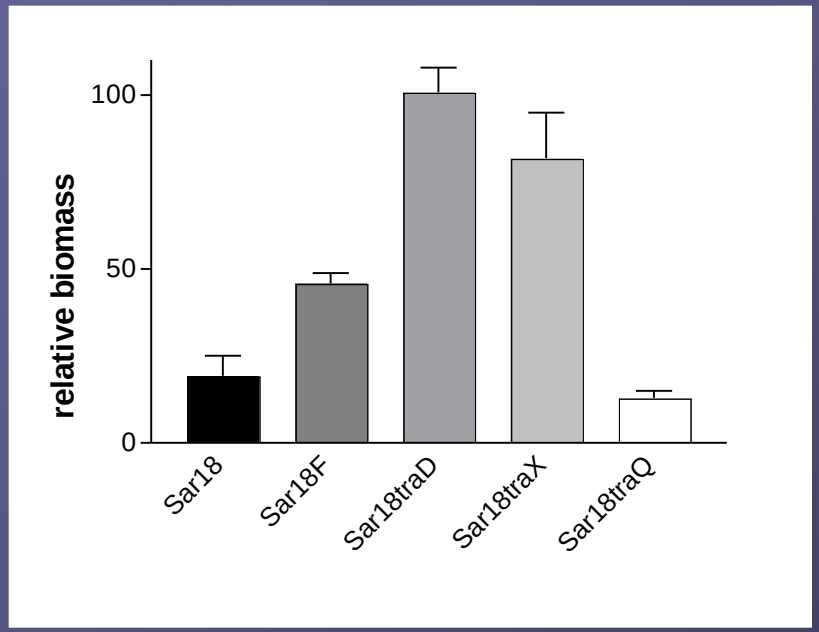
# Differential outcomes after antibiotic challenge in *E.coli* biofilms.



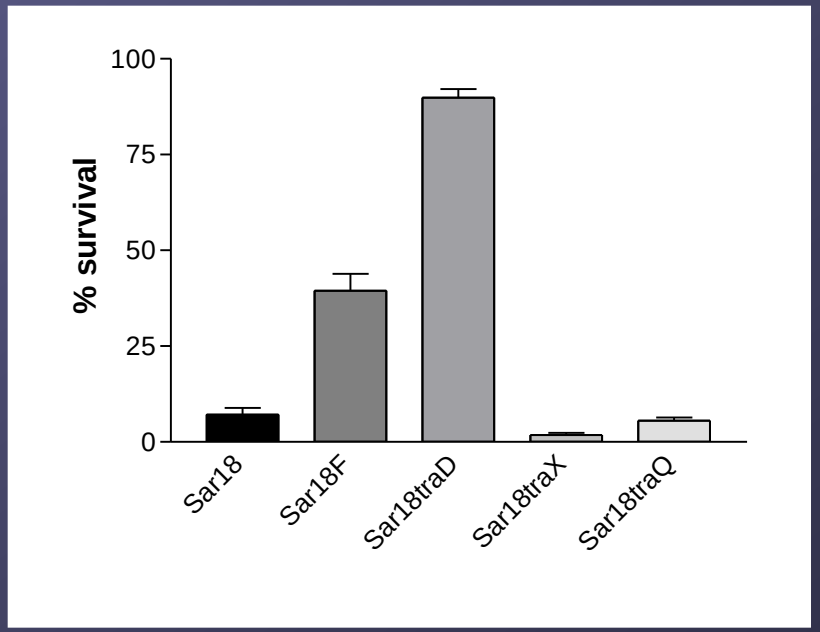

10 x MIC

# Differential survival after colistin challenge in *E.coli* biofilms.

## Biofilm formation (CV stain)



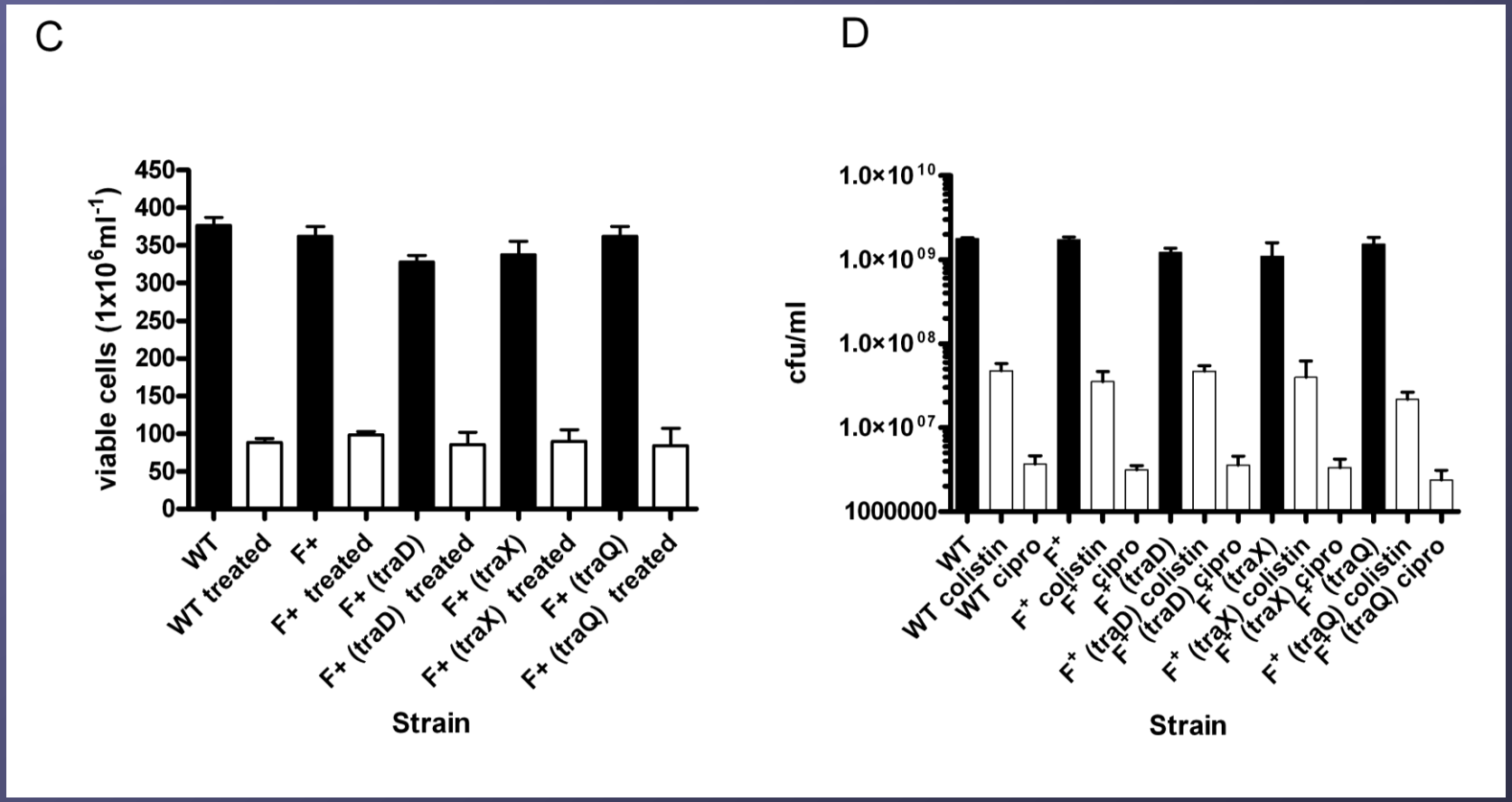

## Survival after treatment



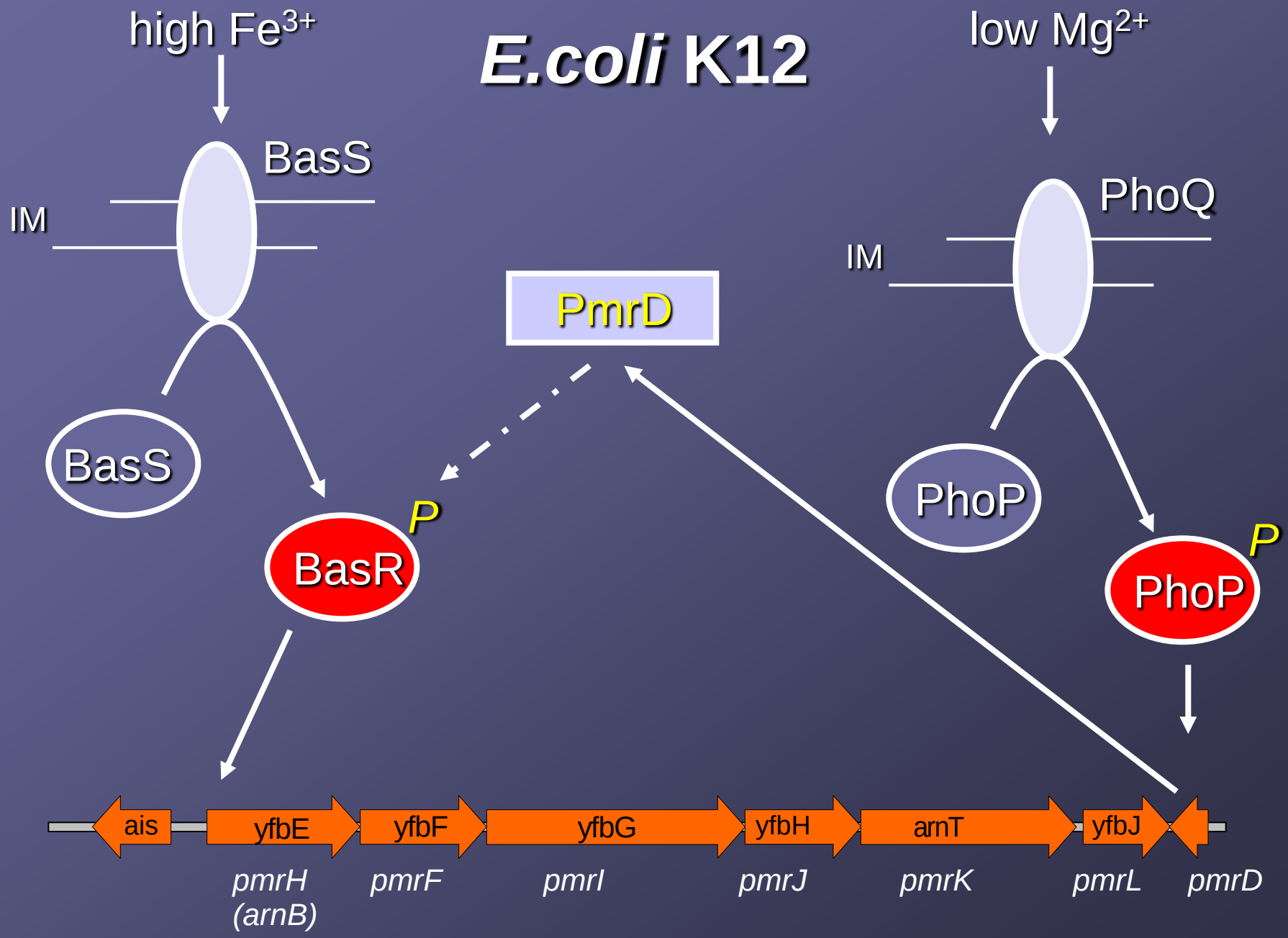
# *E.coli* cells in biofilms differing in organization display differential survival after colistin treatment

Survival Ciprofloxacin

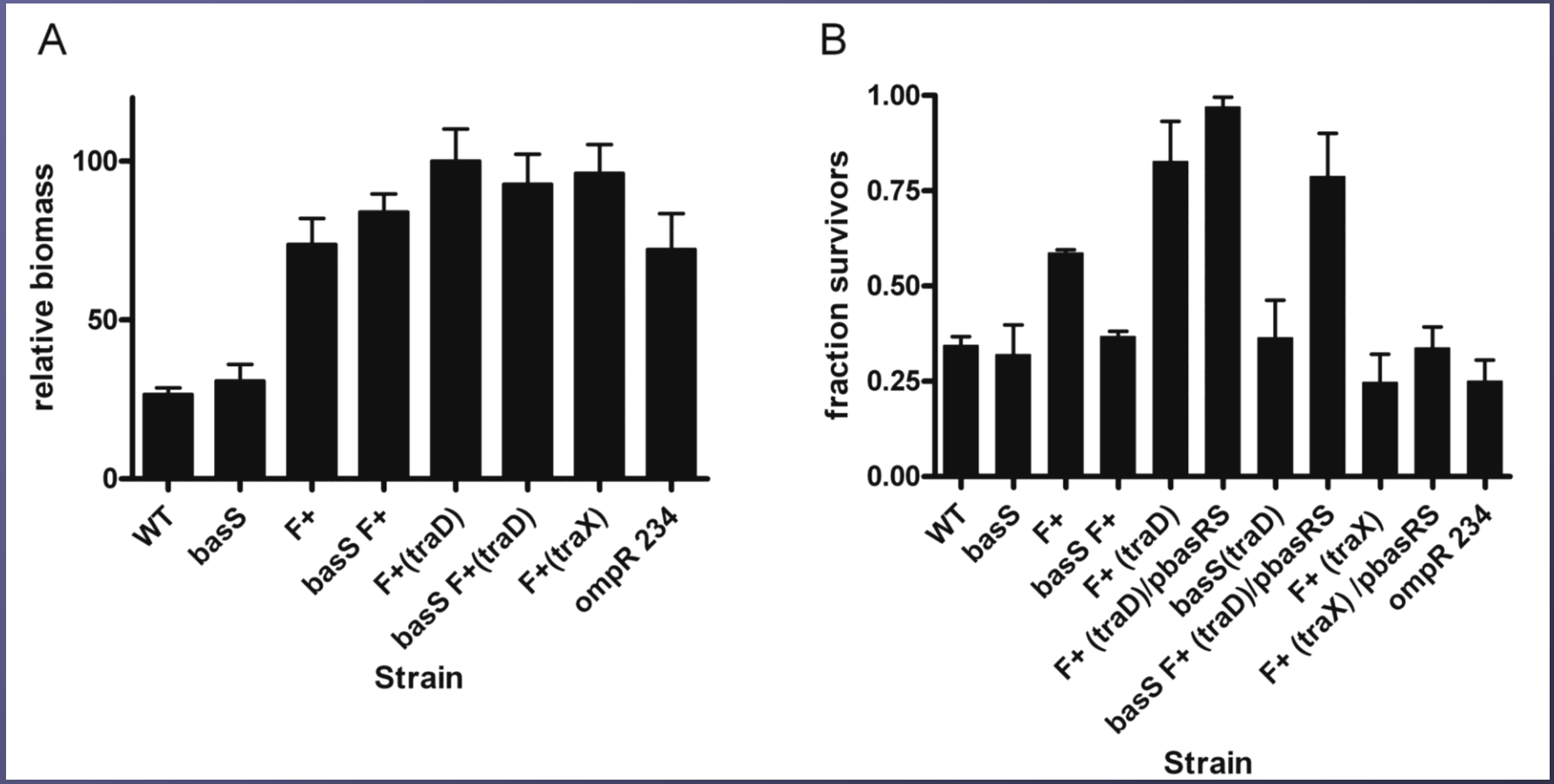
Survival planktonic



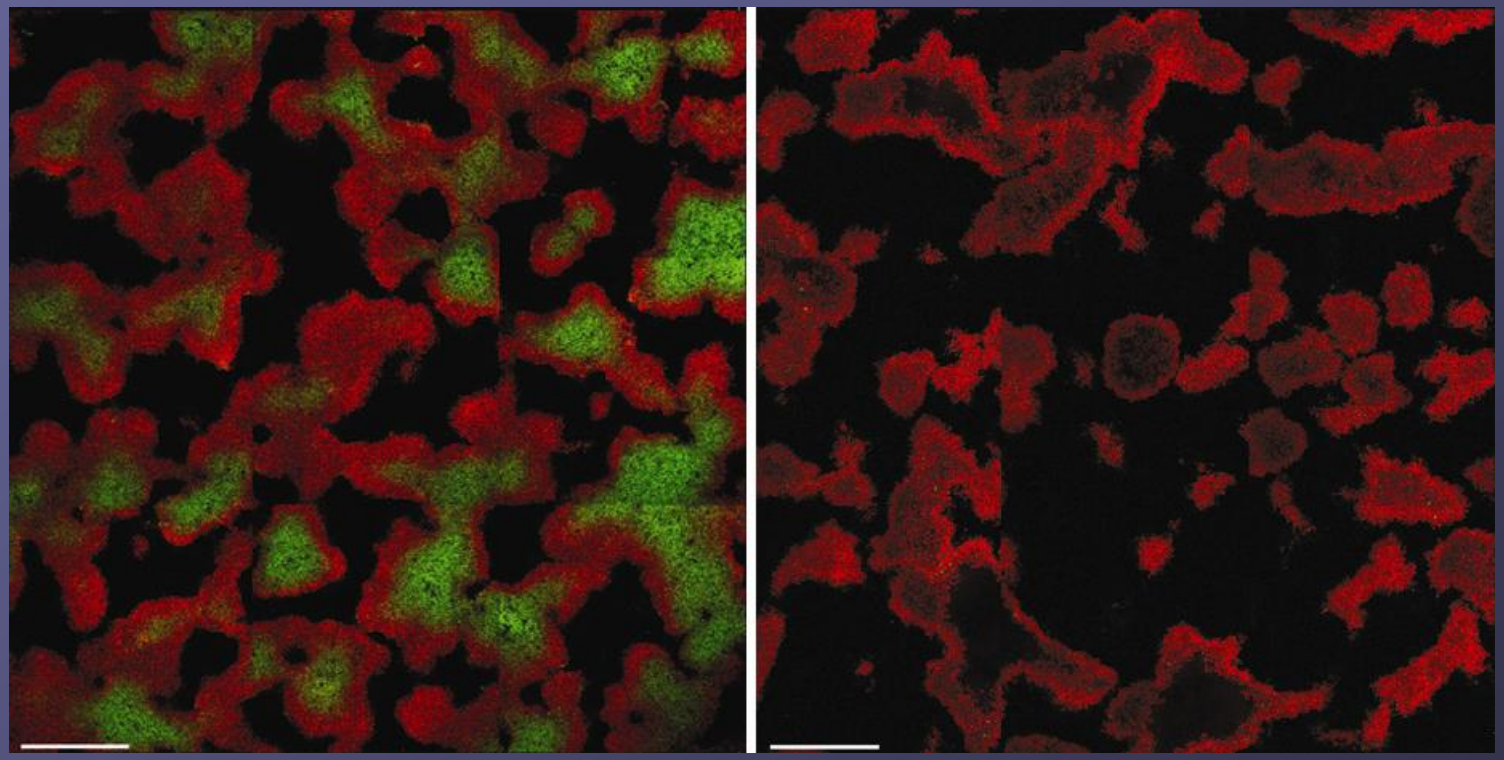
**Is the increased survival due to poor penetration of the antimicrobial peptide or a specific tolerance mechanism?**


high Fe3+
E.coli K12
low Mg2+
BasS
IM
PhoQ
IM
PmrD
BasS
P
BasR
PhoP
PhoP
P
ais
yfbE
yfbF
yfbG
yfbH
arnT
yfbJ
pmrH (arnB)
pmrF
pmrI
pmrJ
pmrK
pmrL
pmrD

# The increased survival is *basS* dependent and not due to poor penetration of the antimicrobial peptide.

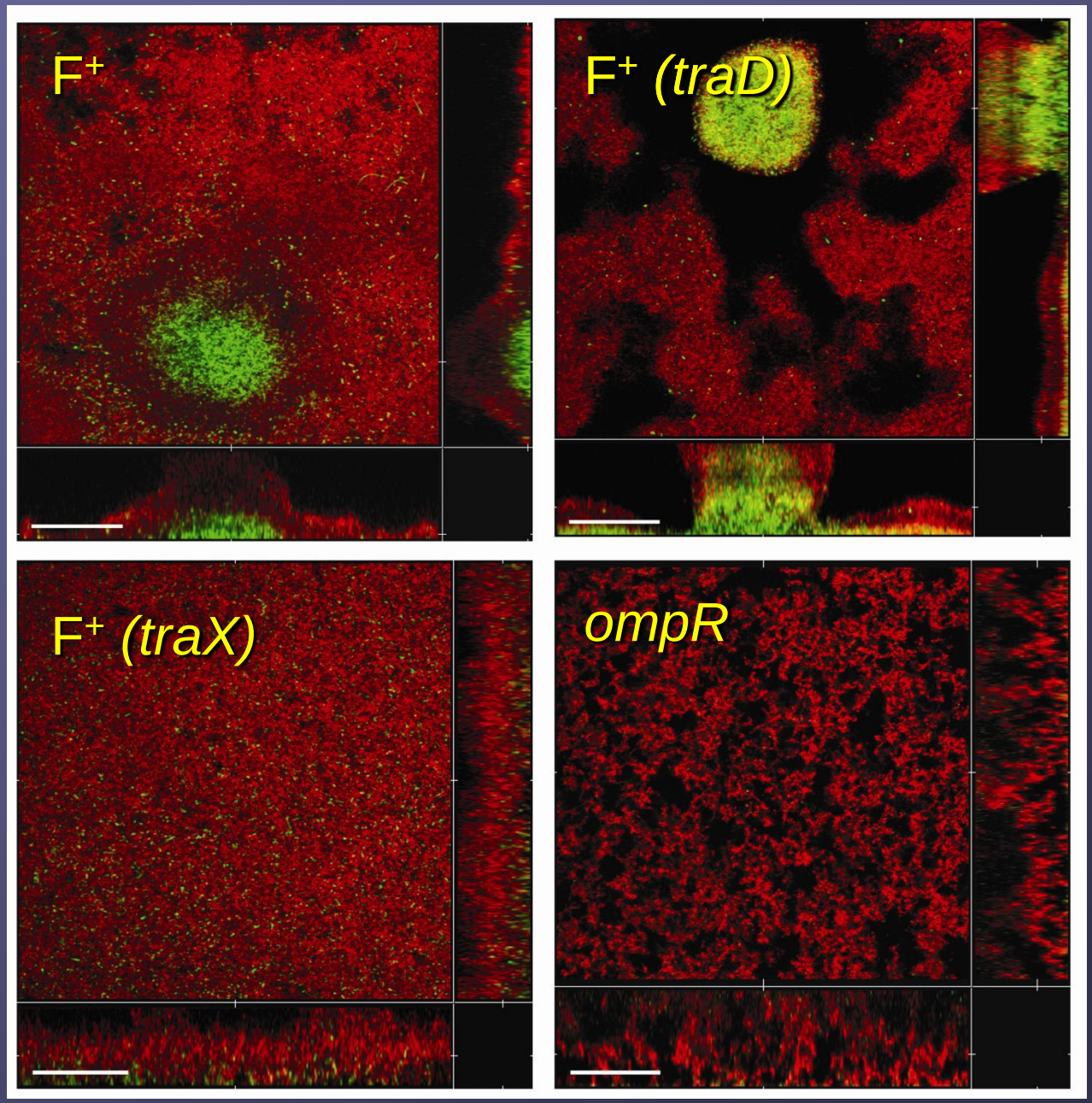


# A colistin tolerant subpopulation is formed in *E.coli* flow-chamber biofilms.

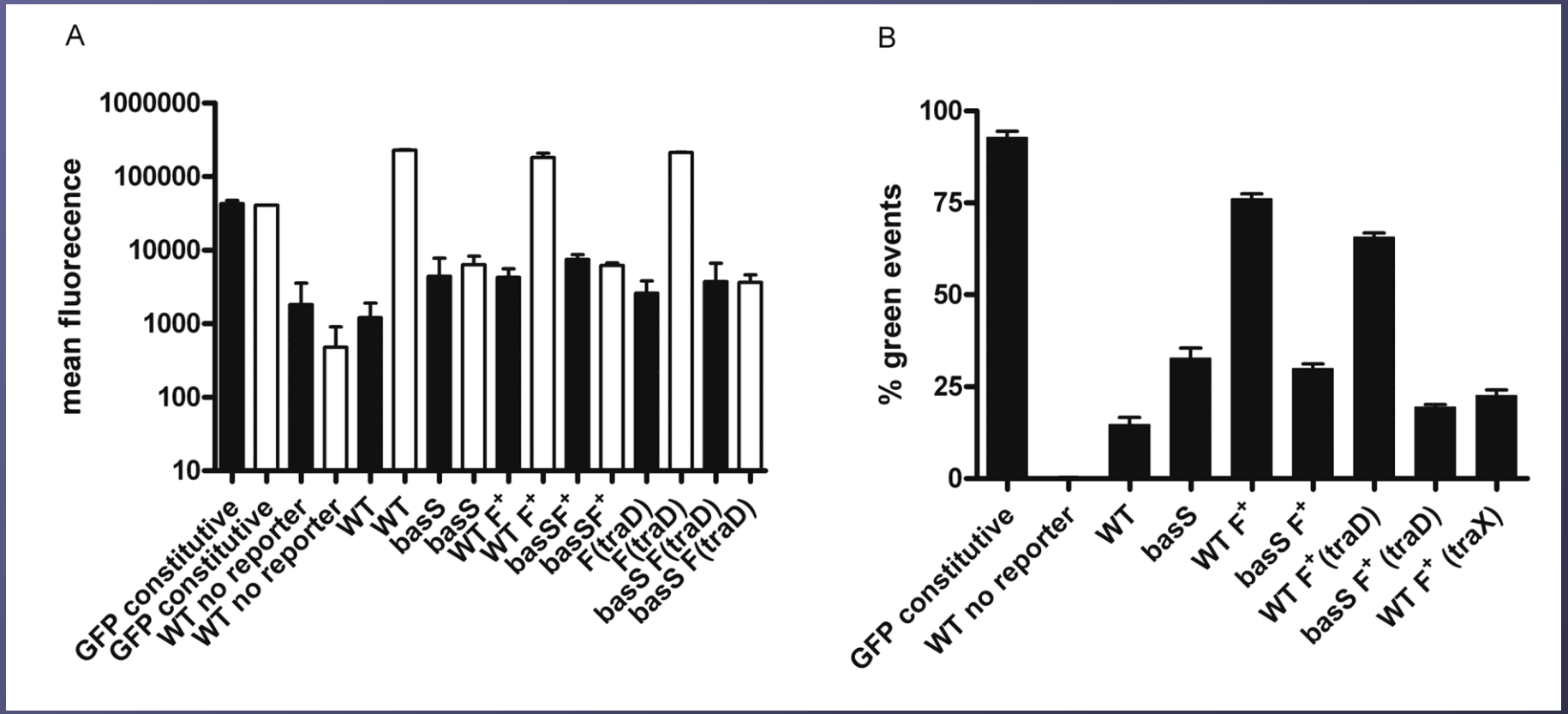


**Biofilm mediated antibiotic tolerance is matter of subpopulation differentiation within the biofilm.**

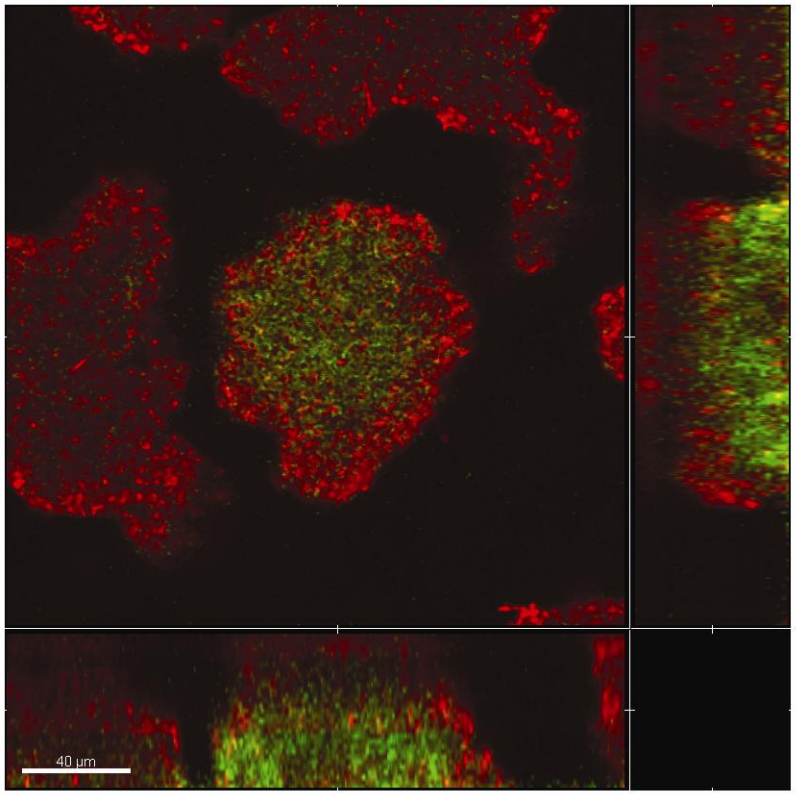
**Colistin tolerance is influenced by biofilm structure and architecture.**

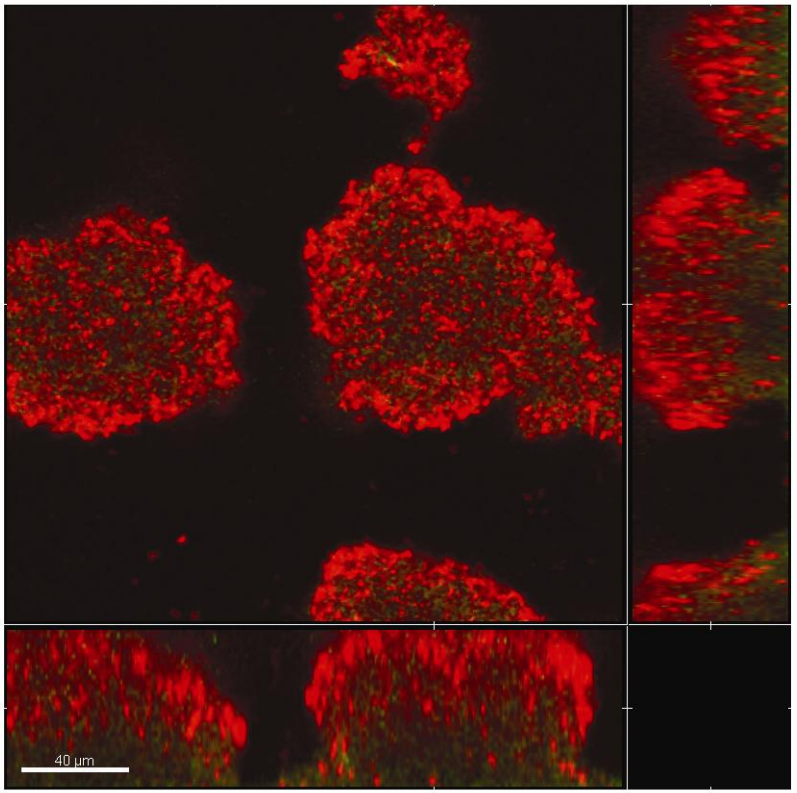


**That this subpopulation depends upon *basR/S* is convincing. What is not so convincing is that this subpopulation is present at the time of addition of colistin.**



# The *yfbE* gene is induced within structured biofilms prior to colistin challenge.



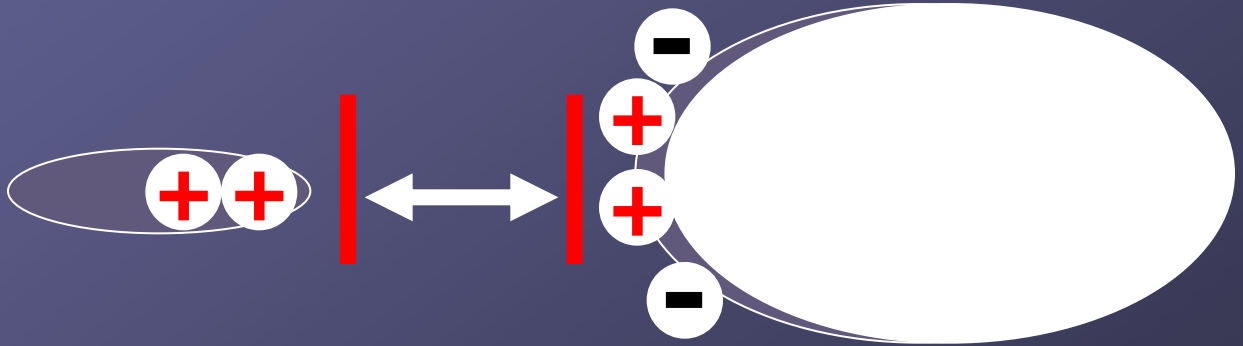
Biofilm mediated antibiotic tolerance is matter of subpopulation differentiation within the biofilm.

The increased antibiotic tolerance exhibited by biofilm formation is **antibiotic specific** and **conditional**, dependent on the actual **biofilm structure** and on the presence of a **specific** genetically encoded **tolerance mechanism**.

The biofilm mode of growth does not directly predict antibiotic resistance

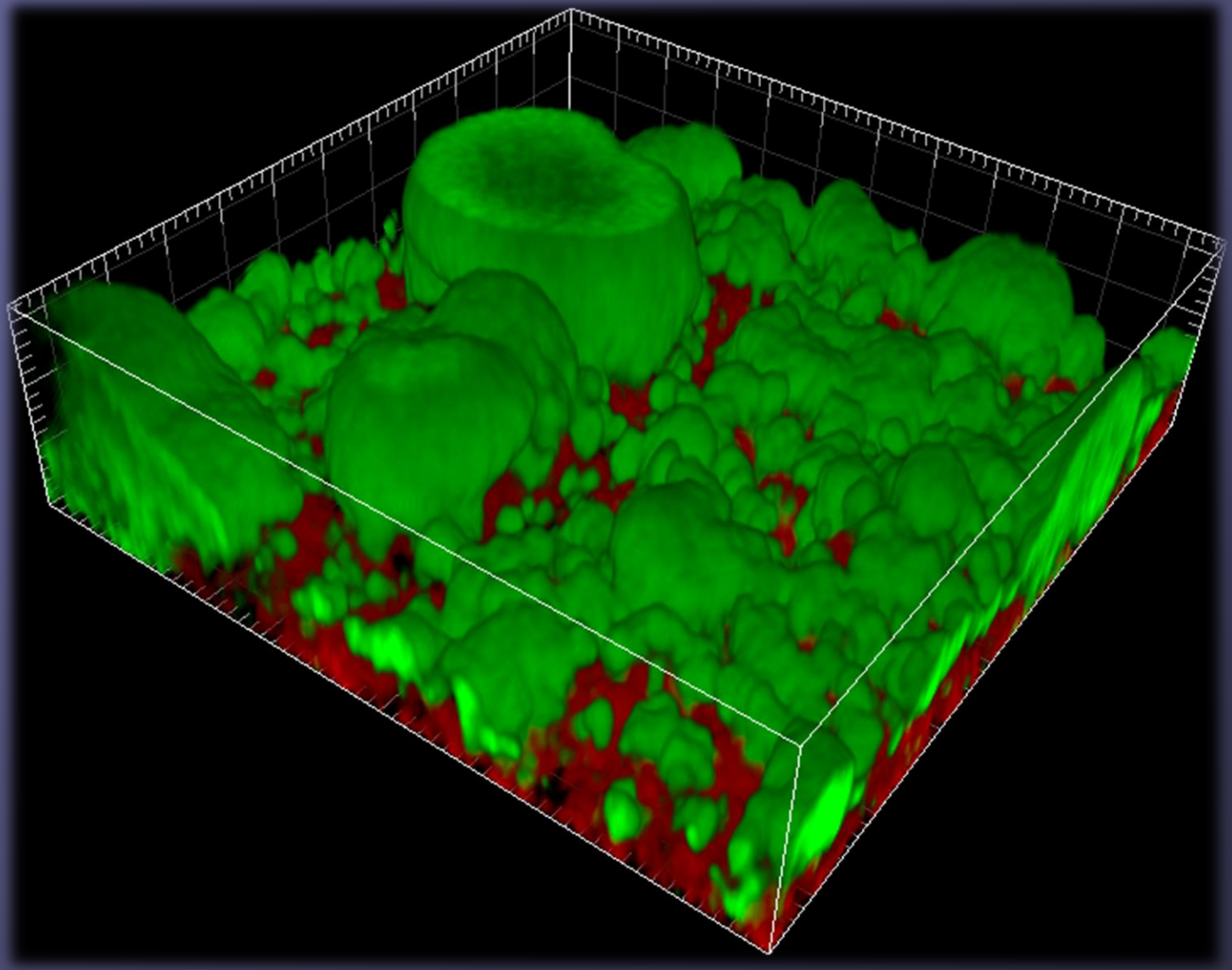
# *P. aeruginosa* can sense and respond to the presence of CAMP.

**Outer-membrane modification**



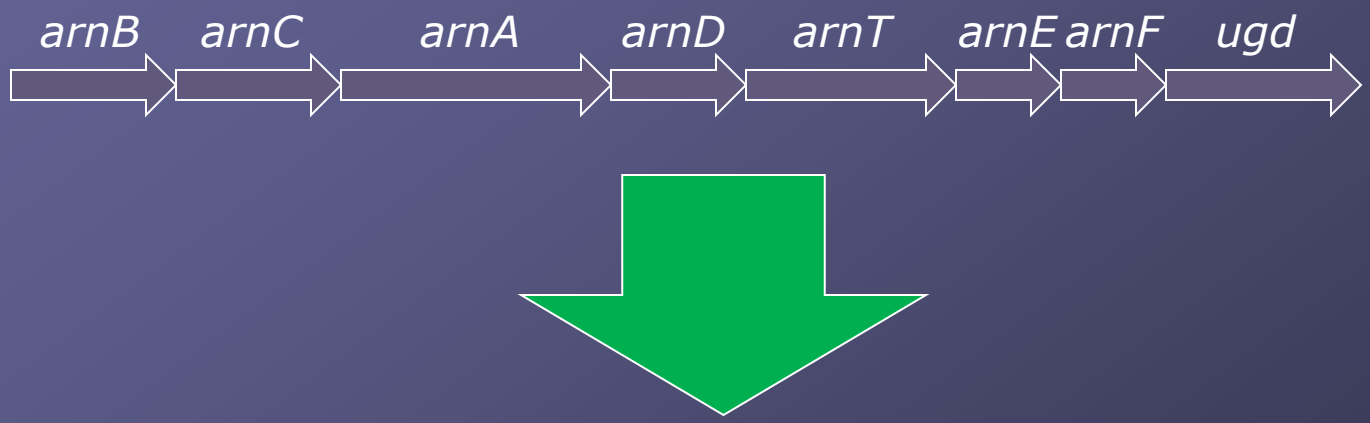
Broad CAMP resistance through mutations in *pmrB*

# A colistin tolerant subpopulation is formed in *P.aeruginosa* flow-chamber biofilms.



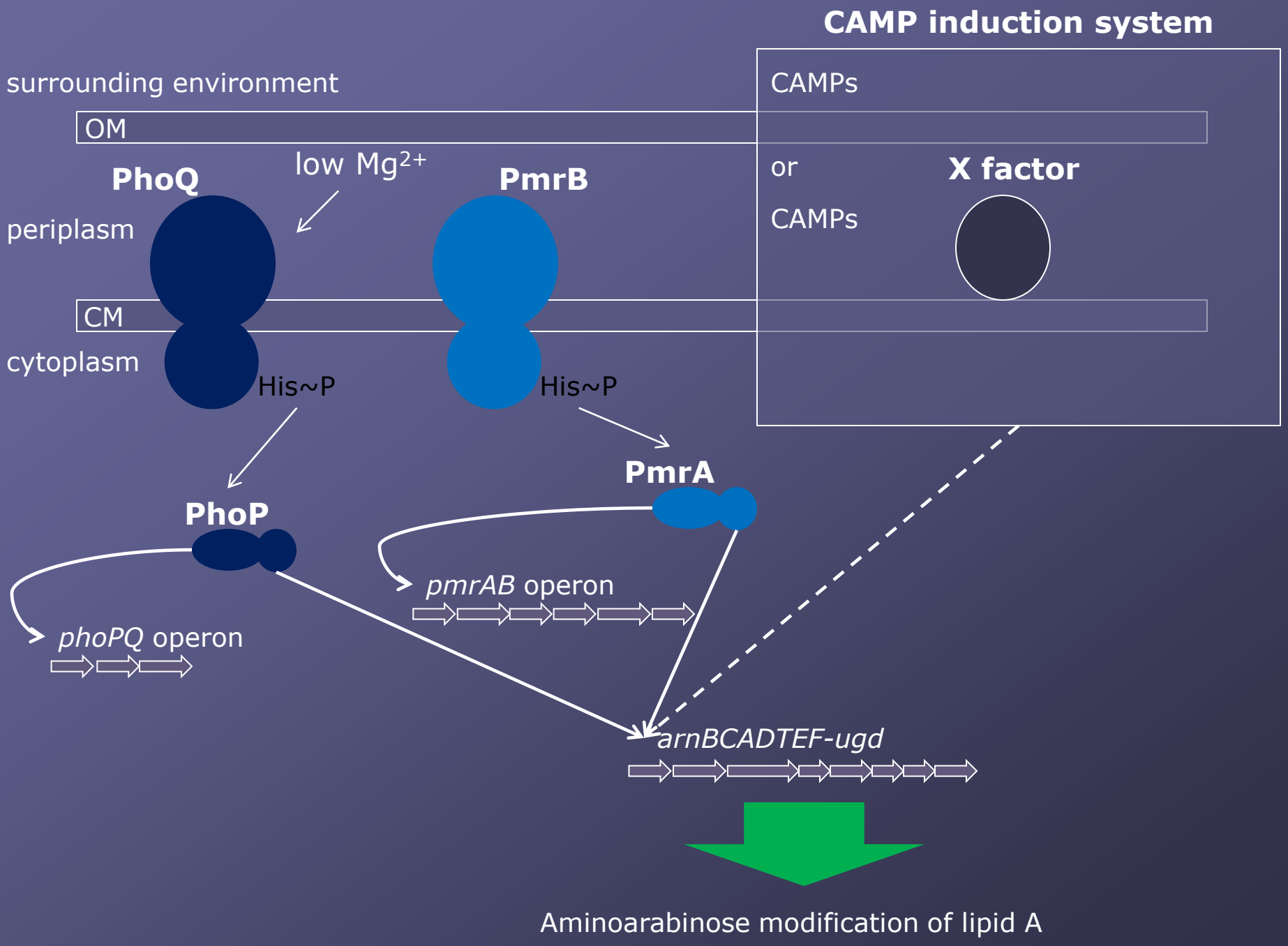
# The *arnB* operon of *P. aeruginosa*

- **It encodes the enzymes necessary for the addition of $N_4$-aminoarabinose to the 4' and 1' phosphates of lipid A in LPS**




$N_4$-aminoarabinose modification of lipid A

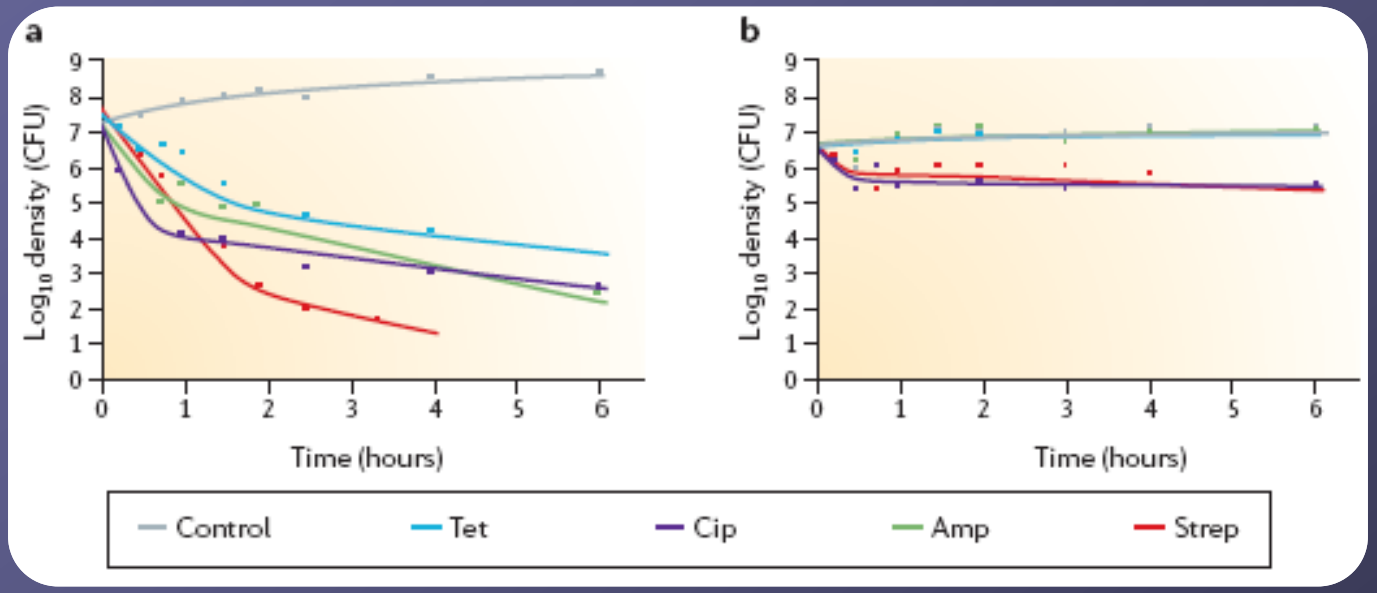
»» increased tolerance towards a broad range of CAMPs


CAMP induction system
surrounding environment
CAMPs
OM
low Mg2+
PhoQ
PmrB
or
X factor
CAMPs
periplasm
CM
cytoplasm
His~P
His~P
PmrA
PhoP
pmrAB operon
phoPQ operon
arnBCADTEF-ugd
Aminoarabinose modification of lipid A
N.Jochumsen 2009

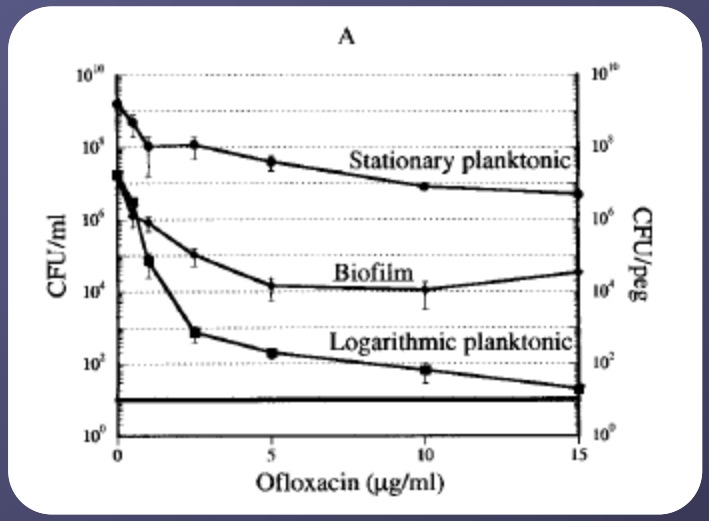
**Although biofilms have some properties in common, their structure and composition depends on the component microorganisms and environmental conditions.Thus, in different situations, the level of antibiotic resistance may vary and the factors that give rise to the increased resistance may differ.**

R. Kolter

# Biofilm vs stationary phase as an explanation for the failure of AB treatment without resistance.




Levin et.al 2006




Spoering et.al 2001

# Further reading:

1-3


1. Monds, R.D. & O'Toole, G.A. The developmental model of microbial biofilms: ten years of a paradigm up for review. *Trends Microbiol* **17**, 73-87 (2009).

2. Moxon, E.R., Sweetman, W.A., Deadman, M.E., Ferguson, D.J. & Hood, D.W. Haemophilus influenzae biofilms: hypothesis or fact? *Trends Microbiol* **16**, 95-100 (2008).

3. Zuroff, T.R. et al. Robustness analysis of culturing perturbations on Escherichia coli colony biofilm beta-lactam and aminoglycoside antibiotic tolerance. *BMC Microbiol* **10**, 185 (2010).

## How about *Pseudomonas aeruginosa*?

**Biofilm systems 4.**

***Haagensen et.al. J.Bact (2007)***

# Aim.

The aim of this exercise is to demonstrate how the hydrodynamic
flow chamber biofilm system can be used to monitor gene expression in heterogeneous populations.
We will use a reporter gene fusion
to the *arnB* and *pmrAB* operon inserted in the att Tn7 site to monitor the
response of cells within the biofilm to colistin challenge.

Moreover, we will use a
isogenic regulatory mutant to visualize differential regulation within structured environments.

There are a few question we would like you to pay extra attention to during the exercise:

Where and how is *arnB* expressed?

Where and how is *pmrAB* expressed?

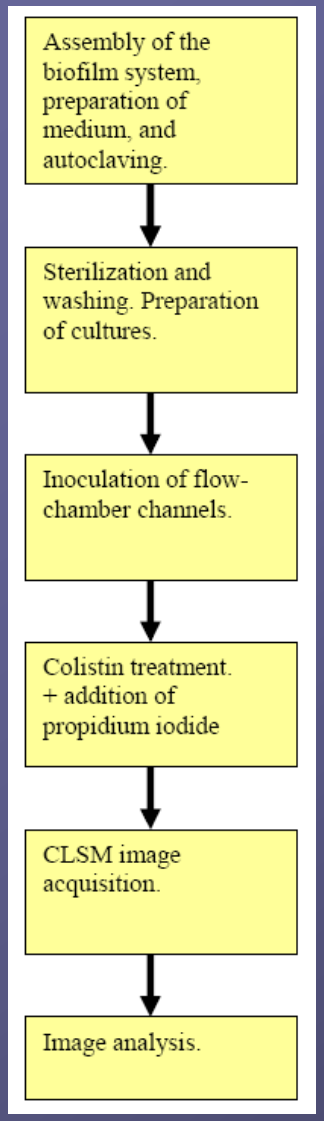
Is there a correlation in *arnB* and *pmrAB* expression?

How does colistin concentration affect the *arnB* and *pmrAB* expression pattern in the biofilms?

What factors are affecting the expression?

What are the strengths and weaknesses of monitoring gene expression flow chamber biofilms?

What are the implications of these results on the study of biofilms and biofilm formation in general?




# Minimal inhibitory concentration

E-test

Serial dilution